1235-Plat
Force Spectrum Microscopy Reveals Active Diffusive-Like Fluctuations in Living Cells
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A living cell is a far-from-equilibrium dynamic material, replete with active force generation due to molecular motors and other enzymatic activity. While motors often produce highly directed motion, the aggregate yet incoherent effect of all active processes can create randomly fluctuating forces that can lead to diffusive-like, but inherently non-thermal motion. These fluctuating forces drive many basic cellular processes, making it essential to characterize their behavior; however, there is no existing technique to measure these forces. Here we introduce a new assay to quantify the random forces directly, by combining measurements of the random motion of probe particles with independent micromechanical measurements of the cytoplasm, to quantitatively determine the spectrum of these active force fluctuations. These active forces substantially enhance intracellular movement over a broad range of length scales. Moreover, we show that this force spectrum is a sensitive assay to probe motor activity in cells: The fluctuations are three times larger in malignant cancer cells than in their benign counterparts.

1236-Plat
Myosin Light Chain Kinase Activity Regulates the Number of Leading Edges in Zebrafish Embryonic Keratocytes
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Rapidly motile cells such as neutrophils and keratocytes typically polarize to generate a single front and rear in order to sustain productive forward movement. However, other less persistently motile cell types, such as fibroblasts and epithelial cells, frequently have multiple, apparently independent, protrusive fronts. The mechanisms that limit polarization to a single front and rear for rapidly motile cells are largely unknown. Here we use embryonic zebrafish keratocytes to investigate how a purely lamellipodial cytoskeleton self-organizes to regulate protrusion number and overall cell polarity. We have observed that keratocytes derived from 4 days post-fertilization (dpf) embryos often form multiple protrusions, suggesting that the motility machinery in these cells polarizes differently from "single front" keratocytes such as those found at 2dpf. To elucidate the mechanism for this difference in polarization behavior, we performed gene expression analysis on 2dpf and 4dpf cells and found that 4dpf cells express higher levels of MLCK. Treatment of 4dpf cells with the MLCK inhibitor ML7 can induce ~50% of multiple-front cells to form a single leading edge. To determine whether ML7 induces single-front behavior by decreasing actin turnover, we tested whether ML7 treatment synergizes with the actin filament stabilizing drug jasplakinolide, and found that >90% will form a single front in response to this drug combination. We have also found that ML7 treatment increases the width of leading edges in 4dpf cells, including those that don't form a single front. Based on these results, we speculate that increased MLCK activity in 4dpf cells increases actin turnover at the leading edge, limiting the size of each protrusion. This represents a novel mechanism for myosin activity to regulate cell polarity not by controlling the rear but rather by influencing the front from afar.

1237-Plat
Quantitative Subcellular Control of Cdc42, Rac1 and RhoA GTPases using the Cry2/CIBN Optogenetic Dimerizer
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Cell polarization is established and maintained through complex mechanisms involving signaling networks regulated in space and time at the sub-cellular level. How a cell coordinates multiple local signaling modules to create a global polarization is still unclear. Recently developed optogenetic methods have been recognized as promising tools to dissect these intracellular signaling networks by allowing perturbations to be spatially and temporally controlled. First, we characterized the biophysical properties of the Cry2/CIBN light gated dimerization system in order to establish the quantitative relationship between patterns of light stimulation and corresponding gradients of induced signaling activity. We analyzed the processes involved in the recruitment and localization of Cry2 including the 3D cytoplasmic diffusion of Cry2, the 2D diffusion of Cry2/CIBN complexes at the cell membrane and the disassociation kinetics of this complex. From these experiments we determined that it is possible to induce subcellular gradients of recruited proteins of any chosen profile up to a spatial resolution of 5µm and a temporal one of ca. 3 minutes. Second, we applied our quantitative optogenetic method to the regulation of Cdc42, Rac1 and RhoA, the three canonical RhoGPTases involved in cell polarity and migration. We quantified the effect of the local activation of Cdc42 using cell displacement and cell shape changes as reporters of cell polarization and migration. We qualitatively characterized the effects of local activation of RhoA and Rac1 on different cellular effectors including actin filaments and focal adhesion complexes. Altogether, our quantitative optogenetic method provides a new step for the optogenetic dissection of subcellular signaling networks by allowing the simultaneous measurement of the perturbation and the cell response in a straightforward and reproducible way.

Platform: Bioengineering

1238-Plat
Biomechanical Basis of Alzheimer's Disease and Other Protein Misfolding Diseases: Designing a New AFM Probe to Study Amyloid-Mediated Membrane Disorders
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Most important biological interactions with its environment occur at interfaces such as a lipid cellular membrane. These interactions are often dynamic and show time-dependent changes in material properties (e.g, viscoelastic properties). A detailed understanding of biomechanical properties at molecular and subcellular level is vital for basic understanding of normal/abnormal biological processes. Indeed, abnormal changes in the mechanics of biological systems are often indicators of pathophysiological states. For example, amyloid beta peptide (Aβ), a protein present in neural plaques formed in Alzheimer's disease and cholesterol are reported to alter cell membrane mechanical properties leading to neuronal degeneration. Atomic force microscopy (AFM) provides an ideal tool to image the structure and examine the mechanics in physiologically relevant medium. We have created a new cantilevered probe for AFM for the study of nano-to-microscale mechanics of biological interfaces. We used it to examine the role of Aβ1-42 insertion in DPPC lipid monolayers and its effect on the viscoelastic properties that underlie altered membrane fluidity. Real-time analysis of mechanical properties of the model membrane monolayer in the presence of Aβ1-42 shows a decrease in the viscosity of the monolayers over time, consistent with increased membrane fluidity. The finding is consistent with changes in membrane permeability due to Aβ-inserted pores.

1239-Plat
Light-Powered Bionanoelectronic Devices with Biologically-Tunable Performance Characteristics
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Bacteriorhodopsin (bR) is a well-characterized photoactivated proton pump capable of creating a proton gradient using the energy of absorbed light. We are developing a bioelectronic platform that couples bacteriorhodopsin activity with the electronic response of a nanowire field-effect transistor. To accomplish this task we use hierarchical assembly of lipids and membrane proteins into a 1-D lipid bilayer structure covering the channel region of the transistor device. This presentation discusses the device preparation, characterization, and overall performance. In addition, we present results showing how other ion channels and ionophores, co-assembled with the protein embedded bilayer, can be used to change various performance characteristics of the device, independent of one-another. The ability to use components of the biological "toolkit" to fine-tune bioelectronic devices could have important implications for design of next generation biointerfaces.

1240-Plat
Light Driven Conformational Switching: An Approach to Creating Designed Protein Motion
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Biomolecular motors have inspired the design and construction of artificial nanoscale motors and machines based on nucleic acids, small molecules, and inorganic nanostructures. However, the high degree of sophistication and efficiency of biomolecular motors derives from the complexity afforded by protein building blocks. Here, we discuss a novel bottom-up approach to understanding biological motors and present a class of designs for synthetic protein motors that move along a linear DNA track.

This presentation will focus on two aspects of the motor design. Firstly the use of self-assembling components whose function is to co-localise the necessary motor functions. We demonstrate that a coiled coil template can be adapted in order to program the self-assembly of three different coiled coils from a solution of six peptides.

Secondly the role of linkers between components in controlling dynamics and hence both processivity and power strokes within our motor designs will be discussed. The use of cis-trans isomerization of azobenzene as a mechanism for driving conformational change within the motor will be demonstrated.

1. The Tumbleweed: towards a synthetic protein motor, EHC Bromley et al. HFSP J. 3, 204-212 (2009).
2. Tuning the performance of an artificial protein motor, NJ Kuwada et al. Phys. Rev. E 84(3) 031922 (2011).
3. Designed α-Helical Tectons for Constructing Multicomponent Synthetic Biological Systems EHC Bromley et al. J. Am. Chem. Soc. 131, 928-930 (2009).
4. Squaring the circle in peptide assembly: From fibers to discrete Nanostructures by de Novo Design. AL Boyle et al. J. Am. Chem. Soc. 134, 15457-15467 (2012).

1241-Plat

High Throughput Live-Cell FRET Binding Assay by Flow Cytometry

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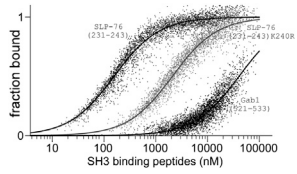
Ratiometric live-cell fluorescence resonance energy transfer (FRET) assays hold significant promise for measuring binding affinity of protein-protein interactions. Compared to conventional *in vitro* binding assays, live-cell FRET gauges interaction within a native regime that favors proper folding, permits physiological modulation of association, and obviates costly purification of proteins. Previously, our lab developed a live-cell FRET binding assay (three-cube method) for cell-by-cell epifluorescence and confocal microscopy (*Neuron***31**:973, *J Microsc***233**: 192). While accurate (*Nat Commun***4**:1717), this approach is comparatively slow and labor intensive, challenging high-throughput application. Here we transformed this live-cell FRET approach into the realm of flow cytometry, allowing full binding curves encompassing data from thousands of cells within seconds. We were able to get similar results between the new configuration and microscope-based setup. As an example, we characterized well-studied interactions between Venus-tagged SH3 domains of Gad and a suite of Cerulean-tagged binding peptides. Three such interactions are illustrated with a range of affinities, in close agreement with *in vitro* iTC measurements. This new flow-cytometry approach (3^3-FRET-HTS) opens new possibilities for profiling protein-protein interactions in the native context.



1242-Plat

Quantifying Cell-Surface Marker Expression Through Imaging of Transient Interactions

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Characterizing cells in terms of their surface-marker expression is an indispensable tool for cell biology, disease diagnosis, and drug discovery. Flow cytometry, the most commonly used method, though invaluable, requires exogenous labelling of cells, is not tractable for small sample sizes, and is limited in the number of unique markers that can be measured simultaneously. Recently, node-pore sensing (NPS) has emerged as a label-free method for surface-marker characterization. In NPS, the Coulter principle is utilized to measure the transit time of cells as they flow through a microfluidic channel functionalized with antibodies for multiple surface-markers of interest. Cells transiting functionalized regions of the channel that express the corresponding surface-marker have longer transit times when compared against a control region due to transient and specific interactions with the functionalized surface. In this manner, NPS can screen label-free for multiple surface markers at the single-cell level. The sensitivity of NPS is dependent on the ratio of the cell's volume to the volume of the channel, which limits the length of the channel and thus the number of possible unique markers. Furthermore, since the current through the channel is dependent on its total resistance, only one cell may be present in the channel at a time. Here, we show that these limitations can be overcome if the transit time is measured optically rather than electrically. Imaging the channel allows for tracking individual cells, which enables multiple cells to be measured simultaneously. Moreover, the total number of markers is only limited by the size and speed of the CCD camera that is used for imaging. We utilize this approach to measure the surface-marker expression profile of single MCF-7 and SKBR3 cells.

1243-Plat

Sorting Bacterium Cells Using Cell-Imprinted Polymer Thin Films: From Concept to Applications

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Cell imprinting is a recently developed technology that captures the structural and chemical information on the surface of cells on a polymer surface through template-assisted assembly of functional groups. A polymer is cured around template cells that are removed subsequently, leaving complementary cavities that not only spatially fit but also chemically recognize the target cells {*ACS Nano*, 6: 4314, 2012}. The cell-imprinted materials thereby function as artificial receptors, which are considerably less expensive to produce and more durable than natural receptors and could potentially be broadly used for cell sorting. One promising potential application is the detection of pathogens causing infectious diseases. However, the involvement of pathogens in the production of the imprinted material as well as in the cell capturing process brings occupational risk of infection. Most recently, we discovered that inactivated bacteria can be selectively captured by a polymer film imprinted with the bacteria inactivated in the same way, avoiding the use of live virulent bacteria; moreover, the inactivation strategies, especially those utilizing chemical reagents, resulted in better selectivity of capture than when living cells were used {*ACS Nano*, 7: 6031, 2013}. This inactivation process may have played two roles: (1) to eliminate the secretion of extracellular matrix, which helps expose the surface of the cells during imprinting; (2) to fix the cells, which helps preserve the structural and chemical information on their surface. Utilizing a cell-imprinted thin film, we are developing a rapid, culture-free, low-cost and high-sensitivity diagnosis for tuberculosis infection, which may fulfill the significant demand of effective and affordable diagnosis for tuberculosis in low-income countries. Also, we are developing an inexpensive cell-imprinted wipe for rapid detection of bio-contamination on solid surfaces, which may have broad applications in food safety control and public security.

1244-Plat

Patient-Specific iPSCs-Based Liver-On-A-Chip

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Successful advent of future medicine (e.g., personalized, preventive, predictive, participating medicine) will be strongly bonded to the availability of human-physiology-describing models. The combination of advanced researches in induced personalized stem cells (iPSCs) and the state-of-the-art microengineering is considered to create in-vitro models of organ functions in a near future; the first can generate patient-specific human cell lines, and the latter enables physiologically relevant microenvironments for recapitulating organ-level functions. Here we present a patient-specific iPSC-derived hepatocytes in an integrated microphysiological analysis platform (iMAP). The iMAP for iPSC-derived hepatocyte model is designed to culture the hepatocytes using endothelial-like physical barriers, which separate cell culture chambers and perfusion channels with Peclet number $<<1$. The physiological architecture supports enhanced hepatic functions of the iPSCs-derived hepatocytes, especially protein synthesis and drug metabolism. Compared to a conventional sandwich culture method, the liver-on-a-chip supports three-dimensional sinusoidal organization, enhanced junction protein and continuously refreshed media around the hepatic tissue to express higher albumin secretion and specifically enhanced drug metabolism (e.g., phenacetin is more metabolized to acetaminophen and N-acetyl-p-benzoquinone imine). Also, iPSCs-derived hepatocytes, by being matured from hepatoblast in the liver-on-a-chip platform, can increase the structural integrity due to avoiding dissociation of polarized hepatocytes and organogenesis-like tissue development in the microenvironment. The iMAP for iPSC-derived hepatocyte model will be tested for patient-specific pharmacokinetics/pharmacodynamics by involving various patient-derived iPSCs, and also other organ models will be integrated to estimate organ-organ interactions, which will provide various human-physiology-describing in-vitro models.

1245-Plat

A Biophysical Solution to Prevent Hemolysis Interferences

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In-vivo and *in-vitro* hemolysis, membrane rupture of red blood cells (RBCs) and release of their contents into blood plasma, is one of the major critical issues in medicine. For *in-vitro* blood-based molecular diagnostics, inhibitors (hemoglobin, potassium, mRNA etc.) released from RBCs due to hemolysis during blood plasma separation can cause serious difficulties in quantitative